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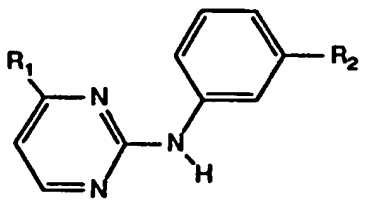
(54) Title: PYRIMIDINEAMINE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

(57) Abstract

N-phenyl-2-pyrimidineamine derivatives of formula (I) wherein the substituents are as defined in claim 1 are described. Those compounds can be used, for example, in the treatment of tumour diseases.



(I)

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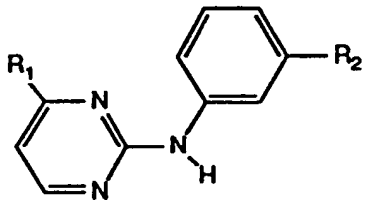
Pyrimidineamine derivatives and processes for the preparation thereof

The invention relates to N-phenyl-2-pyrimidineamine derivatives, to processes for the preparation thereof, to medicaments comprising those compounds, and to the use thereof in the preparation of pharmaceutical compositions for the therapeutic treatment of warm-blooded animals.

The invention relates to N-phenyl-2-pyrimidineamine derivatives of formula I



(I)

wherein

R_1 is naphthyl, fluorenyl, anthracenyl or a substituted cyclic radical, the cyclic radical being bonded to a ring carbon atom in each case and being selected from phenyl, pyridyl, 1H-indolyl, pyrazinyl, thiazolyl, pyrimidinyl, pyridazinyl and imidazolyl, and the substituents of the above-mentioned phenyl radical being selected from hydroxy, halogen, nitro, cyano, unsubstituted or halogen-substituted lower alkoxy, from a radical of formula II

$$-C(=O)-(O)_m-R_3 \qquad (II)$$

wherein

m is 0 or 1 and

R_3 is hydrogen, benzyl, lower alkyl or amino-lower alkyl wherein the amino group is free, lower alkylated or lower alkanoylated,

from a radical of formula III

$$-C(=O)-N(R_4)R_5 \qquad (III)$$

wherein

R_4 and R_5 are each independently of the other hydrogen or unsubstituted or amino- or

hydroxy-substituted lower alkyl,
from a radical of formula IV

$$-SO_2-N(R_6)R_7 \qquad (IV)$$

wherein
R_6 and R_7 are each independently of the other hydrogen, lower alkyl or amino-lower alkyl, or wherein
R_6 and R_7 together form the bivalent radical $-(CH_2)_2-NH-(CH_2)_2-$,
and from a radical of formula V

$$-N(R_8)R_9 \qquad (V)$$

wherein
R_8 and R_9 are each independently of the other lower alkyl, or wherein
R_8 is hydrogen and
R_9 is amino or amino-cyclohexyl, or is lower alkyl that is substituted by imidazolyl, guanidyl, lower alkylamino-carbonylamino, amidino, di-lower alkylamino-cyclohexyl, piperazinyl, carboxy, lower alkoxycarbonyl, carbamoyl, N-hydroxy-carbamoyl, hydroxy, lower alkoxy, dihydroxyphosphoryloxy or by formylpiperazinyl,
and the substituents of the other above-mentioned cyclic radicals being selected from hydroxy, halogen, cyano, amino-lower alkyl, unsubstituted or halogen-substituted lower alkoxy, phthalimido-substituted lower alkyl, from a radical of the above-mentioned formulae II, III or IV and from a radical of formula VI

$$-N(R_{10})R_{11} \qquad (VI)$$

wherein
R_{10} and R_{11} are each independently of the other hydrogen or lower alkyl, or wherein
R_{10} is hydrogen and
R_{11} is amino or amino-cyclohexyl, or is lower alkyl substituted by amino, lower alkylamino, di-lower alkylamino, lower alkanoylamino, imidazolyl, guanidyl, lower alkylamino-carbonylamino, amidino, di-lower alkylamino-cyclohexyl, piperazinyl, formylpiperazinyl, carboxy, lower alkoxycarbonyl, carbamoyl, N-hydroxy-carbamoyl, hydroxy, lower alkoxy, dihydroxyphosphoryloxy or by glycylamido; and
R_2 is nitro, fluorine-substituted lower alkoxy or a radical of formula VII

$$-N(R_{12})-C(=X)-(Y)_n-R_{13} \qquad (VII)$$

wherein
R_{12} is hydrogen or lower alkyl,
X is oxo, thio, imino, N-lower alkyl-imino, hydroximino or O-lower alkyl-hydroximino,
Y is oxygen or the group NH,
n is 0 or 1, and
R_{13} is an aliphatic radical having at least 5 carbon atoms, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical,
and to salts of such compounds having at least one salt-forming group.

Naphthyl R_1 is 1-naphthyl or 2-naphthyl.

Anthracenyl R_1 is preferably 9-anthracenyl.

Fluorenyl R_1 is preferably 2-fluorenyl.

A substituted phenyl radical R_1 can have several substituents, but especially not more than 3 and, especially in the case of relatively large substituents, preferably only one substituent, which substituents are principally in the *para*- (or 4-position) and/or preferably *meta*-position (or 3-position). The other above-mentioned substituted cyclic radicals R_1 generally have up to two and preferably only one substituent, which is especially in the *meta*- or *para*-position with respect to the bonding site of the cyclic radical R_1.

Pyridyl bonded to a ring carbon atom is 2- or preferably 4- or 3-pyridyl, especially 4-pyridyl. In a mono-substituted pyridyl radical R_1, the substituent is preferably in the *ortho*-position with respect to the pyridine nitrogen.

1H-indolyl bonded to a carbon atom of the five-membered ring is 1H-indol-2-yl or preferably 1H-indol-3-yl. In mono-substituted 1H-indolyl, the substituent is preferably in the 1-position, that is to say, at the nitrogen.

Halogen in a radical R_1 is preferably chlorine or fluorine.

Halogen-substituted phenyl R_1 is preferably 2-, 3- or 4-chloro-phenyl, 2,4-, 3,4- or 2,5-dichloro-phenyl or 2,3,4-trichloro-phenyl.

Unsubstituted or halogen-substituted lower alkoxy as substituent of a substituted phenyl radical R_1 is preferably methoxy, ethoxy or trifluoro-methoxy.

A radical of formula II is, for example, a radical wherein m is 1 and R_3 is hydrogen, that is to say, carboxy.

A radical of formula III is, for example, a radical wherein R_4 is hydrogen and R_5 is hydrogen, ω-amino-alkyl having 2 or 3 carbon atoms or ω-hydroxy-alkyl having 2 or 3 carbon atoms, that is to say, carbamoyl, 2-amino-ethyl, 2-hydroxy-ethyl, 3-amino-propyl or 3-hydroxy-propyl.

A radical of formula IV is, for example, a radical wherein R_6 is hydrogen and R_7 is ω-amino-C_{2-3}alkyl or wherein R_6 and R_7 together form the bivalent radical $-(CH_2)_2-NH-(CH_2)_2-$, that is to say, they form a piperazinyl ring together with the nitrogen atom to which R_6 and R_7 are bonded.

Amino-cyclohexyl R_9 or R_{11} is preferably 4-amino-cyclohexyl. Di-lower alkylamino-cyclohexyl as the radical R_9 or as part of a substituted lower alkyl radical R_9 or R_{11} is preferably 4-di-lower alkylamino-cyclohexyl, preferably 4-dimethylamino-cyclohexyl.

Lower alkylamino in a radical R_9 or R_{11} is preferably methylamino.

Di-lower alkylamino in a radical R_9 or R_{11} is preferably dimethylamino.

Lower alkanoylamino in a radical R_9 or R_{11} is preferably acetylamino.

Formyl-piperazinyl in a radical R_9 or R_{11} is preferably 4-formyl-piperazinyl.

Lower alkyl R_9 substituted by imidazolyl, guanidyl, lower alkylamino-carbonylamino, amidino, di-lower alkylamino-cyclohexyl, piperazinyl, carboxy, lower alkoxycarbonyl, carbamoyl, N-hydroxy-carbamoyl, hydroxy, lower alkoxy, dihydroxyphosphoryloxy or by formylpiperazinyl, or lower alkyl R_{11} substituted by amino, lower alkylamino, di-lower alkylamino, lower alkanoylamino, imidazolyl, guanidyl, lower alkylamino-carbonylamino,

amidino, di-lower alkylamino-cyclohexyl, piperazinyl, formylpiperazinyl, carboxy, lower alkoxycarbonyl, carbamoyl, N-hydroxy-carbamoyl, lower alkoxy, dihydroxyphosphoryl-oxy or by glycylamido is preferably mono-, di- or tri-methylene substituted in that manner, the substituents preferably being in the ω-position. In addition, hydroxy-substituted lower alkyl R_9 or R_{11} is preferably also 2-hydroxy-propyl.

Fluorine-substituted lower alkoxy R_2 is lower alkoxy that carries at least one, but preferably several, fluorine substituents, especially trifluoromethoxy or more especially 1,1,2,2-tetrafluoro-ethoxy.

When X is oxo, thio, imino, N-lower alkyl-imino, hydroximino or O-lower alkyl-hydroximino, the group C=X is, in the order mentioned, the radical C=O, C=S, C=N-H, C=N-lower alkyl, C=N-OH or C=N-O-lower alkyl. X is preferably oxo.

n is preferably 0, that is to say, the group Y is not present.

Y, if present, is preferably the group NH.

Within the scope of this text, the term "lower" denotes radicals having up to and including 7, preferably up to and including 4, carbon atoms.

Unless otherwise indicated in the context concerned, lower alkyl is preferably methyl or ethyl.

An aliphatic radical R_{13} having at least 5 carbon atoms preferably has not more than 22 carbon atoms and generally not more than 10 carbon atoms and is such a substituted or preferably unsubstituted aliphatic hydrocarbon radical, that is to say, such a substituted or preferably unsubstituted alkynyl, alkenyl or preferably alkyl radical, such as C_5-C_7alkyl, for example n-pentyl. An aromatic radical R_{13} has up to 20 carbon atoms and is unsubstituted or substituted, for example naphthyl, such as especially 2-naphthyl, or preferably phenyl, each of which is unsubstituted or substituted, the substituents preferably being selected from cyano, lower alkyl that is unsubstituted or substituted by hydroxy, amino or by 4-methyl-piperazinyl, such as especially methyl, from trifluoromethyl, free, etherified or esterified hydroxy, free, alkylated or acylated amino and from free or esterified carboxy. In an aromatic-aliphatic radical R_{13}, the aromatic moiety is as defined above and the aliphatic moiety is preferably lower alkyl, such as especially C_1-C_2alkyl, that is subs-

tituted or preferably unsubstituted, for example benzyl. A cycloaliphatic radical R_{13} has especially up to 30, principally up to 20 and more especially up to 10 carbon atoms, is mono- or poly-cyclic and is substituted or preferably unsubstituted, for example such a cycloalkyl radical, especially a 5- or 6-membered cycloalkyl radical, such as preferably cyclohexyl. In a cycloaliphatic-aliphatic radical R_{13}, the cycloaliphatic moiety is as defined above and the aliphatic moiety is preferably lower alkyl, such as especially C_1-C_2-alkyl that is substituted or preferably unsubstituted. A heterocyclic radical R_{13} contains especially up to 20 carbon atoms and is preferably a saturated or unsaturated monocyclic radical having 5 or 6 ring members and from 1 to 3 hetero atoms which are preferably selected from nitrogen, oxygen and sulfur, especially, for example, thienyl or 2-, 3- or 4-pyridyl, or a bi- or tri-cyclic radical, wherein, for example, one or two benzene radicals are fused (annellated) to the mentioned monocyclic radical. In a heterocyclic-aliphatic radical R_{13}, the heterocyclic moiety is as defined above and the aliphatic moiety is preferably lower alkyl, such as especially C_1-C_2alkyl, that is substituted or preferably unsubstituted.

Etherified hydroxy in a radical R_{13} is preferably lower alkoxy. Esterified hydroxy in a radical R_{13} is preferably hydroxy esterified by an organic carboxylic acid, such as a lower alkanoic acid, or by a mineral acid, such as a hydrohalic acid, for example lower alkanoyl-oxy or especially halogen, such as iodine, bromine or especially fluorine or chlorine.

Alkylated amino in a radical R_{13} is, for example, lower alkylamino, such as methylamino, or di-lower alkylamino, such as dimethylamino. Acylated amino is, for example, lower alkanoylamino or benzoylamino.

Esterified carboxy in a radical R_{13} is, for example, lower alkoxycarbonyl, such as methoxycarbonyl.

Salt-forming groups in a compound of formula I are groups or radicals having basic or acidic properties. Compounds having at least one basic group or at least one basic radical, for example a free amino group, a pyrazinyl radical or a pyridyl radical, can form acid addition salts, for example with inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid, oxalic acid or amino acids, such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxy-

benzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxy-ethane-sulfonic acid, or aromatic sulfonic acids, for example benzene-, *p*-toluene- or naphthalene-2-sulfonic acid. If several basic groups are present, mono- or poly-acid addition salts can be formed.

Compounds of formula I having acidic groups, for example a free carboxy group in the radical R_1, can form metal or ammonium salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, for example triethylamine or tri(2-hydroxyethyl)amine, or heterocyclic bases, for example N-ethyl-piperidine or N,N'-dimethyl-piperazine.

Compounds of formula I that possess both acidic and basic groups can form internal salts.

For the purpose of isolation or purification and also in the case of the compounds used further as intermediates, it is also possible to use pharmaceutically unacceptable salts. Only the pharmaceutically acceptable non-toxic salts are used therapeutically, however, and those are therefore preferred.

In view of the close relationship between the novel compounds in free form and in the form of their salts, including also salts that can be used as intermediates, for example in the purification of the novel compounds or in order to identify those compounds, hereinbefore and hereinafter any reference to the free compounds is to be understood as including also the corresponding salts, where appropriate and expedient.

The compounds of formula I exhibit valuable pharmacological properties: for example, they inhibit the enzyme protein kinase C with a high degree of selectivity. Phospholipid- and calcium-dependent protein kinase C occurs in cells in a number of forms and participates in various fundamental processes, such as signal transmission, proliferation and differentiation, and also the release of hormones and neurotransmitters. The activation of that enzyme is effected either by receptor-mediated hydrolysis of phospholipids of the cell membrane or by direct interaction with certain tumour-promoting active substances. The sensitivity of the cell to receptor-mediated signal transmission can be substantially influenced by modifying the activity of protein kinase C (as a signal transmitter).

Compounds that are capable of influencing the activity of protein kinase C can be used as tumour-inhibiting, antiinflammatory, immunomodulating and antibacterial active ingredients and may even be of value as agents against atherosclerosis and disorders of the cardiovascular system and central nervous system.

Formerly, porcine brain protein kinase C purified in accordance with the procedure described by T. Uchida and C.R. Filburn in J. Biol. Chem. 259, 12311-4 (1984) was used to determine the inhibitory action on protein kinase C, and the inhibitory action on protein kinase C was determined in accordance with the procedure of D. Fabbro *et al.*, Arch. Biochem. Biophys. 239, 102-111 (1985).

The porcine brain protein kinase C formerly used is a mixture of various sub-types (isotypes) of protein kinase C. If pure recombinant isotypes are used instead of porcine brain protein kinase C in the above test it is found that the compounds of formula I inhibit the "conventional" isotype α preferentially whereas the other "conventional" isotypes β-1, β-2 and γ and especially the "non-conventional" isotypes δ, ϵ and η and the "atypical" isoform ζ are inhibited to a distinctly lesser extent and in some cases hardly at all.

Recombinant PKC isotypes are cloned, expressed and purified in the following manner:

The production of various proteins with the aid of baculoviruses, and their cloning and isolation from Sf9 insect cells are carried out as described by M.D. Summers and G.E. Smith, "A manual method for baculovirus vectors and insect cell culture procedure", Texas Agricul. Exptl. Station Bull. (1987), 1555. The construction and isolation of recombinant viruses for the expression of PKC-α (bovine), PKC-β1 (human), PKC-β2 (human) and PKC-γ (human/bovine hybrid) in Sf9 cells are effected in the manner described by Stabel *et al.* [S. Stabel, M. Liyanage and D. Frith, "Expression of protein kinase C isozymes in insect cells and isolation of recombinant proteins", Meth. Neurosc. (1993)]. The production of the PKC isotypes in Sf9 cells is carried out in the manner indicated by Stabel *et al.* (see above), and the purification of the enzymes is effected in accordance with the method described in the publication by McGlynn *et al.* [E. McGlynn, J. Liebetanz, S. Reutener, J. Wood, N.B. Lydon, H. Hofstetter, M. Vanek, T. Meyer and D. Fabbro, "Expression and partial characterization of rat protein kinase C-δ and protein kinase C-ζ in insect cells using recombinant baculovirus", J. Cell. Biochem. 49, 239-250 (1992)]. For the generation of recombinant PKC-δ (rat), PKC-ϵ (rat), PKC-ζ (rat) and PKC-η (mouse), and their expression and purification, the procedure described by

Liyanage *et al.* ["Protein kinase C group B members PKC-δ, -ε, -ζ and PKC-λ: Comparison of properties of recombinant proteins *in vitro* and *in vivo*", Biochem. J. 283, 781-787 (1992)] and McGlynn *et al.*, respectively, (see above) is followed, with the additional feature that the transfer vector pAc360 is used for the expression of PKC-η [V. Luckow and M.D. Summers, "Trends in the development of baculovirus expression", Biotechnology 6, 47-55 (1988)].

The measurement of the activity of the recombinant PKC isotypes obtained by the above method is carried out in the absence of lipid and calcium (co-factors). Protamine sulfate phosphorylated in the absence of co-factors is used as the substrate. The activity of the enzymes reflects the transfer of ^{32}P from γ-[^{32}P]-ATP to protamine sulfate. Protamine sulfate is a mixture of polypeptides each comprising four C-terminal arginine residues. Phosphate incorporation is measured under the following conditions: 100 µl of the reaction mixture comprise in final concentrations 20 mM TRIS-HCl pH 7.4, 10 mM $Mg[NO_3]_2$, 0.5 mg/ml of protamine sulfate, 10 µM ATP (0.1 µCi γ-[^{32}P]-ATP; 10 Ci/mol; Amersham, Little Chalfont, United Kingdom), various concentrations of the inhibitory compounds and 0.5-2.5 U (units: a unit is the amount of enzyme that, in one minute and per milligram of protein, transfers one nanomole of ^{32}P from the above-mentioned γ-[^{32}P]-ATP to histone H1 [Sigma, type V-S]) of the enzymes. The reaction is started by the addition of the enzymes and transfer at 32°C. The reaction time is 20 minutes. The reaction is then stopped by dripping aliquots of 50 µl onto P81 chromatography paper (Whatman, Maidstone, United Kingdom). After removing unbound γ-[^{32}P]-ATP and nucleotide fragments by washing operations as described by J.J. Witt and R. Roskoski, "Rapid protein kinase assay using phospho-cellulose-paper absorption", Anal. Biochem. 66, 253-258 (1975), the substrate phosphorylation is determined by scintillation measurement. In that test, the compounds of formula I inhibit the α-isotype of protein kinase C (PKC) at an IC_{50} of as low as approximately from 0.1 to 5.0 µmol/litre, generally approximately from 0.1 to 1.0 µmol/litre. In contrast, the other isotypes of PKC are generally inhibited only at distinctly higher concentrations (i.e. at concentrations up to more than 300 times higher).

As may be expected purely on the basis of the above-described inhibitory action on protein kinase C, the compounds of formula I exhibit antiproliferative properties which can be demonstrated directly in another test described in the following in which the inhibitory action of the compounds of formula I on the growth of human T24 bladder carcinoma cells is determined. Those cells are incubated in Eagle's minimal essential

medium, to which 5% (v/v) foetal calf serum has been added, in a humidified incubator at 37°C and with 5% by volume of CO_2 in the air. The carcinoma cells (1000-1500) are sown in 96-well microtitre plates and incubated overnight under the above-mentioned conditions. The test compound is added in serial dilutions on day 1. The plates are incubated for 5 days under the above-mentioned conditions. During that period the control cultures undergo at least four cell divisions. After incubation, the cells are fixed with 3.3% (w/v) aqueous glutaraldehyde solution, washed with water and stained with 0.05% (weight/volume) aqueous methylene blue solution. After washing, the dye is eluted with 3% (w/v) aqueous hydrochloric acid. The optical density (OD) per well, which is directly proportional to the number of cells, is then measured at 665 nm using a photometer (Titertek multiskan). The IC_{50} values are calculated with a computer system using the formula

$$\frac{OD_{665}\text{ (test) minus }OD_{665}\text{ (start)}}{OD_{665}\text{ (control) minus }OD_{665}\text{ (start)}} \times 100$$

The IC_{50} values are defined as being the concentration of active ingredient at which the number of cells per well at the end of the incubation period is only 50% of the number of cells in the control cultures. In the case of the compounds of formula I, the IC_{50} values so ascertained are approximately from 0.1 to 10 µmol/litre.

The anti-tumour activity of the compounds of formula I can also be demonstrated *in vivo*:

Female Balb/c hairless mice with s.c. transplanted human bladder tumours T24 are used to determine the anti-tumour activity. On day 0, with the animals under peroral forene narcosis, approximately 25 mg of a solid tumour are placed under the skin on the animals' left flank and the small incised wound is closed by means of suture clips. On day 6 after the transplantation, the mice are divided at random into groups of 6 animals and treatment commences. The treatment is carried out for 15 days with peroral or intraperitoneal administration once daily of a compound of formula I in dimethyl sulfoxide/Tween 80/-sodium chloride solution in the various doses. The tumours are measured twice a week with a slide gauge and the volume of the tumours is calculated. In that test, the peroral or intraperitoneal administration of a compound of formula I brings about a marked reduction in the average tumour volume in comparison with the untreated control animals.

On the basis of the properties described, the compounds of formula I can be used especially as tumour-inhibiting active ingredients, for example in the treatment of tumours of the bladder and the skin. When the compounds of formula I are used in the treatment of cancer in combination with other chemotherapeutic drugs, they prevent the development of resistance (multidrug resistance) or eliminate an already existing resistance to the other chemotherapeutic drugs. They are also suitable for the other uses mentioned above for protein kinase C modulators and can be used especially in the treatment of disorders responsive to inhibition of protein kinase C.

Some of the compounds of formula I also inhibit the tyrosine kinase activity of the receptor for the epidermal growth factor (EGF). That receptor-specific enzyme activity plays a key role in signal transmission in a large number of mammalian cells, including human cells, especially epithelial cells, cells of the immune system and cells of the central and peripheral nervous system. In the case of various types of cell, the EGF-induced activation of the receptor-associated tyrosine protein kinase (EGF-R-TPK) is a pre-requisite for cell division and accordingly for the proliferation of a cell population. The addition of EGF-receptor-specific tyrosine kinase inhibitors thus inhibits the replication of those cells.

Inhibition of EGF-receptor-specific tyrosine protein kinase (EGF-R-TPK) can be demonstrated, for example, using the method of E. McGlynn *et al.*, Europ. J. Biochem. 207, 265-275 (1992). The compounds according to the invention inhibit the enzyme activity by 50% (IC50) for example at a concentration of from 0.1 to 10 μM.

The compounds of formula I that inhibit the tyrosine kinase activity of the receptor for the epidermal growth factor (EGF) can accordingly be used, for example, in the treatment of benign or malignant tumours. They are able to bring about tumour regression and to prevent metastatic spread and the growth of micrometastases. They can be used especially in the case of epidermal hyperproliferation (psoriasis), in the treatment of neoplasia of epithelial character, for example mastocarcinoma, and in the case of leukaemia. The compounds can also be used in the treatment of disorders of the immune system and inflammation if protein kinases are involved. Furthermore, those compounds of formula I can be used in the treatment of disorders of the central or peripheral nervous system if signal transmission by protein kinases is involved.

The compounds of formula I and salts of such compounds having at least one salt-forming